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Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression

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ABSTRACT (150 words)

Major depressive disorder (MDD) is a common illness accompanied by considerable morbidity, mortality, costs, and heightened risk of suicide. We conducted a genome-wide association (GWA) meta-analysis based in 135,458 cases and 344,901 control, We identified 44 independent and significant loci. The genetic findings were associated with clinical features of major depression, and implicated brain regions exhibiting anatomical differences in cases. Targets of antidepressant medications and genes involved in gene splicing were enriched for smaller association signal. We found important relations of genetic risk for major depression with educational attainment, body mass, and schizophrenia: lower educational attainment and higher body mass were putatively causal whereas major depression and schizophrenia reflected a partly shared biological etiology. All humans carry lesser or greater numbers of genetic risk factors for major depression. These findings help refine and define the basis of major depression and imply a continuous measure of risk underlies the clinical phenotype.

INTRODUCTION

Major depressive disorder (MDD) is a notably complex and common illness[1]. It is often chronic or recurrent and is thus accompanied by considerable morbidity, disability, excess mortality, substantial costs, and heightened risk of suicide[2-8]. Twin studies attribute approximately 40% of the variation in liability to MDD to additive genetic effects (phenotype heritability, h^2)[9], and h^2 may be greater for recurrent, early-onset, and postpartum MDD[10,11]. GWA studies of MDD have had notable difficulties in identifying individual associated loci[12]. For example, there were no significant findings in the initial Psychiatric Genomics Consortium (PGC) MDD mega-analysis (9,240 cases)[13] or in the CHARGE meta-analysis of depressive symptoms (N=34,549)[14]. More recent studies have proven modestly successful. A study of Han Chinese women (5,303 recurrent MDD cases) identified significant loci[15], a meta-analysis of depressive symptoms (161,460 individuals) identified two loci[16], and an analysis of self-reported major depression identified 15 loci (75,607 cases).

There are many reasons why identifying causal loci for MDD has proven difficult[12]. MDD is probably influenced by many genetic loci each with small effects[17], as are most common diseases[18] including psychiatric disorders[19,20]. Estimates of the proportion of variance attributable to genome-wide SNPs (SNP heritability, h^2_{SNP}) indicate that around a quarter of the h^2 for MDD is due to common genetic variants[21,22], and demonstrate that a genetic signal is detectable in GWA data, implying that larger sample sizes are needed to detect specific loci given their effect sizes. Such a strategy has been proven in schizophrenia studies, the flagship adult psychiatric disorder in genomics research. We thus accumulated clinical, population, and volunteer cohorts[23]. This pragmatic approach takes the view that sample size can overcome heterogeneity to identify risk alleles that are robustly associated with major depression. Potential concerns about combining carefully curated research cohorts with volunteer cohorts were ameliorated via multiple lines of evidence that suggest the results are likely to be applicable to clinical MDD. As discussed more fully below, our analyses have neurobiological, clinical, and therapeutic relevance for major depression.

RESULTS

Cohort analyses: phenotype validation

We identified seven cohorts that used a range of methods to ascertain cases with major depression (described in detail in ***Table 1, Supplementary Tables 1-3***). The methods used by these cohorts were extensively reviewed drawing on the breadth of expertise in the PGC, and we assessed the comparability of the cohorts using genomic data. We use "MDD" to refer to directly evaluated subjects meeting standard criteria for major depressive disorder and use "major depression" where case status was determined using alternative methods as well as to the phenotype from the full meta-analysis.

We evaluated the comparability of the seven cohorts by estimating the common-variant genetic correlations (r_g) between them. These analyses strongly supported the comparability of the seven cohorts (***Supplementary Table 3***) as the weighted mean r_g was 0.76 (SE 0.03). The high genetic correlations between the 23andMeD and other cohorts are notable. While there is no statistical evidence of heterogeneity in the r_g estimates between pairs of cohorts (*P*=0.13),

the estimate is statistically different from 1 which may reflect etiological heterogeneity. This estimate can be benchmarked against the slightly larger weighted mean r_g between schizophrenia cohorts of 0.84 (SE 0.05)[21].

Given the positive evidence of the genetic comparability of these cohorts, we completed a GWA meta-analysis of 9.6 million imputed SNPs in 135,458 MDD and major depression cases and 344,901 controls (***Fig. 1***). There was no evidence of residual population stratification[24] (LD score regression intercept 1.018, SE 0.009). We estimated h^2_{SNP} to be 8.7% (SE 0.004, liability scale, assuming lifetime risk 0.15, ***Supplementary Table 3b*** and ***Supplementary Fig. 1***), and note that this is about a quarter of h^2 estimated from twin or family studies[9]. This fraction is somewhat lower than that of other complex traits[18], and is plausibly due to etiological heterogeneity (and reflecting the mean r_g<1 between cohorts).

To evaluate the impact of combining major depression cohorts that used different ascertainment methods, we undertook a series of genetic risk score (GRS) prediction analyses to demonstrate the validity of our GWA results for clinical MDD (***Fig. 2***). Importantly, the variance explained in out-of-sample prediction increased with the size of the GWA discovery cohort (***Fig. 2a***), with the GRS from the full discovery sample meta-analysis explaining 1.9% of variance in liability (***Fig. 2a***, ***Supplementary Fig. 2***, and ***Supplementary Table 4***). For any randomly selected case and control, GRS ranked cases higher than controls with probability 0.57 (i.e., AUC=0.57), and the odds ratio of MDD for those in the 10th versus 1st GRS decile (OR10) was 2.4 (***Fig. 2b***, ***Supplementary Table 4***). GRS analyses in other disorders (e.g., schizophrenia[25]) have shown that mean GRS increases with clinical severity in cases. We found significantly higher major depression GRS in those with more severe MDD, as measured in different ways (***Fig. 2c***). Last, because around half of the major depression cases were identified by self-report (i.e., diagnosis or treatment for clinical depression by a medical professional), we further evaluated the comparability of the 23andMeD cohort with the other cohorts (full meta-analysis excluding 23andMeD, "FMex23") as detailed in ***Fig. 2c, Supplementary Table 5*** and ***Supplementary Note.*** Taken together, we interpret these results as supporting this meta-analysis of GWA results for these seven cohorts.

Implications of the individual loci for the biology of major depression

Our meta-analysis of seven MDD and major depression cohorts identified 44 independent loci that were statistically significant (P<5x10^{-8}), statistically independent of any other signal[26], and supported by multiple SNPs. This number supports our prediction that GWA discovery in major depression would require about five times more cases than for schizophrenia (lifetime risk ~1% and h^2~0.8) to achieve approximately similar power[27]. Of these 44 loci, 30 are novel and 14 were significant in a prior study of MDD or depressive symptoms. The overlap of our findings with prior reports were: 1/1 with CHARGE depressive symptom[14], 1/2 overlap with SSGAC depressive symptom[16], and 12/15 overlap with Hyde et al.[28]). There are few trans-ancestry comparisons for major depression so we contrasted these European results with the Han Chinese CONVERGE study[15] (***Supplementary Note***). The loci identified in CONVERGE are uncommon in Europeans (rs12415800 0.45 vs 0.02 and rs35936514 0.28 vs 0.06) and were, not significant in our analysis.

Table 2 lists genes in or near the lead SNP in each region, regional plots are in ***Supplementary Data 1***, and ***Supplementary Tables 6-7*** provide extensive summaries of available information about the biological functions of the genes in each region. In the ***Supplementary Note*** we review four key genes in more detail: *OLFM4* and *NEGR1* (notable for reported associations with obesity and body mass index[29-34]), *RBFOX1* (notable for independent our associations at both the 5' and the 3' ends, a splicing regulator[35,36], with a functional role that may be consistent with chronic hypothalamic-pituitary-adrenal axis hyperactivation reported in MDD[37]), and *LRFN5* (notable for its role in pre-synaptic differentiation[38,39] and neuroinflammation[40]).

Gene-wise analyses identified 153 significant genes after controlling for multiple comparisons (***Supplementary Table 7***). Many of these genes were in the extended MHC region (45 of 153) and their interpretation is complicated by high LD and gene density. In addition to the genes discussed above, other notable and significant genes outside of the MHC include multiple potentially "druggable" targets that suggest connections of the pathophysiology of MDD to neuronal calcium signaling (*CACNA1E* and *CACNA2D1*), dopaminergic neurotransmission (*DRD2*, a principal target of antipsychotics), glutamate neurotransmission (*GRIK5* and *GRM5*), and presynaptic vesicle trafficking (*PCLO*).

Finally, comparison of the major depression loci with 108 loci for schizophrenia[19] identified six shared loci. Many SNPs in the extended MHC region are strongly associated with schizophrenia, but implication of the MHC region is novel for

major depression. Another example is *TCF4* (transcription factor 4) which is strongly associated with schizophrenia but not previously with MDD. TCF4 is essential for normal brain development, and rare mutations in *TCF4* cause Pitt–Hopkins syndrome which includes autistic features[41]. GRS calculated from the schizophrenia GWA results explained 0.8% of the variance in liability of MDD (***Fig. 2c***).

Implications from integration of functional genomic data

Results from "-omic" studies of functional features of cells and tissues are necessary to understand the biological implications of results of GWA for complex disorders[42]. To further elucidate the biological relevance of the major depression findings, we integrated the results with a wide range of functional genomic data. First, using enrichment analyses, we compared the major depression GWA findings to bulk tissue mRNA-seq from GTEx[43]. Only brain samples showed significant enrichment (***Fig. 3A***), and the three tissues with the most significant enrichments were all cortical. Prefrontal cortex and anterior cingulate cortex are important for higher-level executive functions and emotional regulation which are often impaired in MDD. Both of these regions were implicated in a large meta-analysis of brain MRI findings in adult MDD cases[44]. Second, given the predominance of neurons in cortex, we confirmed that the major depression genetic findings connect to genes expressed in neurons but not oligodendrocytes or astrocytes (***Fig. 3B***)[45]. Given the different methods used by the seven MDD/major depression cohorts in this study, demonstration of enrichment of association signals in the brain regions expected to be most relevant to MDD provides independent support for the validity of our approach.

Third, we used partitioned LD score regression[46] to evaluate the enrichment of the major depression GWA findings in over 50 functional genomic annotations (***Fig. 3C*** and ***Supplementary Table 8***). The major finding was the significant enrichment of h^2_{SNP} in genomic regions conserved across 29 Eutherian mammals[47] (20.9 fold enrichment, P=1.4x10^{-15}). This annotation was also the most enriched for schizophrenia[46]. We could not evaluate regions conserved in primates or human "accelerated" regions as there were too few for confident evaluation[47]. The other enrichments implied regulatory activity, and included open chromatin in human brain and an epigenetic mark of active enhancers (H3K4me1). Notably, exonic regions did not show enrichment suggesting that, as with schizophrenia[17], genetic variants that change exonic sequences may not play a large role in major depression. We found no evidence that Neanderthal introgressed regions were enriched for major depression GWA findings[48].

Fourth, we applied methods to integrate GWA SNP results with those from gene expression and methylation quantitative trait loci studies (eQTL and mQTL). SMR[49] analysis identified 13 major depression associated SNPs with strong evidence that they control local gene expression in one or more tissues, and nine with strong evidence that they control local DNA methylation (***Supplementary Table 9*** and ***Supplementary Data 2***). A transcriptome-wide association study[50] applied to data from the dorsolateral prefrontal cortex[51] identified 17 genes where major depression-associated SNPs influenced gene expression (***Supplementary Table 10***). These genes included *OLFM4* (discussed above).

Fifth, we added additional data types to attempt to improve understanding of individual loci. For the intergenic associations, we evaluated total-stranded RNA-seq data from human brain and found no evidence for unannotated transcripts in these regions. A particularly important data type is assessment of DNA-DNA interactions which can localize a GWA finding to a specific gene that may be nearby or hundreds of kb away[52-54]. We integrated the major depression results with "easy Hi-C" data from brain cortical samples (3 adult, 3 fetal, > 1 billion reads each). These data clarified three associations. The statistically independent associations in *NEGR1* (rs1432639, P=4.6x10^{-15}) and over 200 kb away (rs12129573, P=4.0x10^{-12}) both implicate *NEGR1* (***Supplementary Fig. 3a***), the former likely due to the presence of a reportedly functional copy number polymorphism (see ***Supplementary Note***) and the presence of intergenic loops. The latter association has evidence of DNA looping interactions with *NEGR1*. The association in *SOX5* (rs4074723) and the two statistically independent associations in *RBFOX1* (rs8063603 and rs7198928, P=6.9x10^{-9} and 1.0x10^{-8}) had only intragenic associations, suggesting that the genetic variation in the regions of the major depression associations act locally and can be assigned to these genes. In contrast, the association in *RERE* (rs159963 P=3.2x10^{-8}) could not be assigned to *RERE* as it may contain super-enhancer elements given its many DNA-DNA interactions with many nearby genes (***Supplementary Fig. 3b***).

Implications based on the roles of sets of genes

A parsimonious explanation for the presence of many significant associations for a complex trait is that the different associations are part of a higher order grouping of genes[55]. These could be a biological pathway or a collection of genes with a functional connection. Multiple methods allow evaluation of the connection of major depression GWA results to sets of genes grouped by empirical or predicted function (i.e., pathway or gene set analysis).

Full pathway analyses are in ***Supplementary Table 11***, and 19 pathways with false discovery rate q-values < 0.05 are summarized in ***Fig. 4***. The major groupings of significant pathways were: RBFOX1, RBFOX2, RBFOX3, or CELF4 regulatory networks; genes whose mRNAs are bound by FMRP; synaptic genes; genes involved in neuronal morphogenesis; genes involved in neuron projection; genes associated with schizophrenia (at $P<10^{-4}$)[19]; genes involved in CNS neuron differentiation; genes encoding voltage-gated calcium channels; genes involved in cytokine and immune response; and genes known to bind to the retinoid X receptor. Several of these pathways are implicated by GWA of schizophrenia and by rare exonic variation of schizophrenia and autism[56,57], and immediately suggest shared biological mechanisms across these disorders.

A key issue for common variant GWA studies is their relevance for pharmacotherapy. We conducted gene set analysis that compared the major depression GWA results to targets of antidepressant medications defined by pharmacological studies[58], and found that 42 sets of genes encoding proteins bound by antidepressant medications were highly enriched for smaller major depression association *P*-values than expected by chance (42 drugs, rank enrichment test $P=8.5\times10^{-10}$). This finding connects our major depression genomic findings to MDD therapeutics, and suggests the salience of these results for novel lead compound discovery for MDD[59].

Implications based on relationships with other traits

Prior epidemiological studies associated MDD with many other diseases and traits. Due to limitations inherent to observational studies, understanding whether a phenotypic correlation is potentially causal or if it results from reverse causation or confounding is generally difficult. Genetic studies now offer complementary strategies to assess whether a phenotypic association between MDD and a risk factor or a comorbidity is mirrored by a non-zero r_g (common variant genetic correlation) and, for some of these, evaluate the potential causality of the association given that exposure to genetic risk factors begins at conception.

We used LD score regression to estimate r_g of major depression with 221 psychiatric disorders, medical diseases, and human traits[22,60]. ***Supplementary Table 12*** contains the full results, and ***Table 3*** holds the r_g values with false discovery rates < 0.01. First, the r_g were very high between our major depression GWA results and those from two studies of current depressive symptoms. Both correlations were close to +1 (the samples in one report overlapped partially with this meta-analysis[16] but the other did not [14]).

Second, we found significant positive genetic correlations between major depression and every psychiatric disorder assessed along with smoking initiation. This is the most comprehensive and best-powered evaluation of the relation of MDD with other psychiatric disorders yet published, and these results indicate that the common genetic variants that predispose to MDD overlap substantially with those for adult and childhood onset psychiatric disorders, although they remain substantially distinct as well.

Third, the common-variant genetic architecture of major depression was positively correlated with multiple measures of sleep quality (daytime sleepiness, insomnia, and tiredness). The first two of these correlations used UK Biobank data with people endorsing major depression, other major psychiatric disorders, shift workers, and those taking hypnotics excluded. This pattern of correlations combined with the importance of sleep and fatigue in major depression (two criteria for MDD) suggests a close and potentially profound mechanistic relation. Major depression also had a strong genetic correlation with neuroticism (a personality dimension assessing the degree of emotional instability); this is consistent with the literature showing a close interconnection of MDD and this personality trait. The strong negative r_g with subjective well-being underscores the capacity of major depression to impact human health.

Finally, major depression had significant negative genetic correlations with data from two studies of educational attainment, which while often considered at the genetic level as proxy measures of intelligence also likely includes more complex personality constructs. With this in mind, it is relevant to note that the r_g between major depression and IQ[61] was not significantly different from zero, despite an the r_g between years of education and IQ of 0.7, implying complex relationships between these traits worthy of future investigation. We also found significant positive correlations with multiple measures of adiposity, relationship to female reproductive behavior (decreased age at menarche, age at first birth, and increased number of children), and positive correlations with coronary artery disease and lung cancer.

We used bi-directional Mendelian randomization (MR) to investigate the relationships between four traits genetically correlated with major depression: years of education (EDY)[62], body mass index (BMI)[29], coronary artery disease (CAD)[63], and schizophrenia[19]. These traits were selected because all of the following were true: phenotypically associated with MDD, significant r_g with MDD, and >30 independent genome-wide significant associations from large GWA. We report GSMR[64] results but obtained qualitatively similar results with other MR methods (***Supplementary Table 13*** and ***Supplementary Fig. 4***). MR analyses provided evidence for a 1.12-fold increase in major depression per standard deviation of BMI (P_{GSMR}=1.2x10^{-7}) and a 0.84-fold decrease in major depression per standard deviation of EDY (P_{GSMR}=2.3x10^{-6}). There was no evidence of reverse causality of major depression for BMI (P_{GSMR}=0.53) or EDY (P_{GSMR}=0.11). For BMI there was some evidence of pleiotropy, as six BMI SNPs were excluded by the HEIDI-outlier test including SNPs near *OLFM4* and *NEGR1*. Thus, these results are consistent with EDY and BMI as either causal risk factors or correlated with causal risk factors for major depression. These results provide hypotheses for future research to understand these potentially directional relationships.

For CAD, the MR analyses were not significant when considering major depression as an outcome (P_{GSMR}=0.30) or as an exposure (P_{GSMR}=0.12), however, the high standard error of the estimates using MDD SNP instruments implies this analysis should be revisited when more major depression genome-wide significant SNP instruments become available from future GWA studies.

We used MR to investigate the relationship between major depression and schizophrenia. Although major depression had positive r_g with many psychiatric disorders, only schizophrenia has sufficient associations for MR analyses. We found significant bi-directional correlations in SNP effect sizes for schizophrenia loci in major depression (P_{GSMR}=1.1x10^{-40}) and for major depression loci in schizophrenia (P_{GSMR}=1.5x10^{-11}). These results suggest that the major depression-schizophrenia r_g of 0.34 is consistent with partially shared biological pathways being causal for both disorders. Although it is plausible that diagnostic misclassification/ambiguity (e.g., misdiagnosis of MDD as schizoaffective disorder) could contaminate these analyses, levels of misclassification would need to be implausibly high (30% unidirectional, 15% bidirectional) to result in an r_g of ~0.3[REF65].

All MR analyses were repeated after excluding the 23andMeD cohort, and the pattern of results was the same (***Supplementary Table 13***).

DISCUSSION

The nature of severe depression has been discussed for millennia[66]. This GWA meta-analysis is among the largest ever conducted in psychiatric genetics, and provides a body of results that help refine and define the fundamental basis of major depression.

In conducting this meta-analysis of major depression, we employed a pragmatic approach by including cohorts that met empirical criteria for sufficient genetic and phenotypic similarity. Our approach was cautious, clinically informed, guided by empirical data, and selective (e.g., we did not include cohorts with bipolar disorder (which requires MDD), depressive symptoms, neuroticism, or well-being). Approximately 44% of all major depression cases were assessed using traditional methods (PGC29, GenScot), treatment registers (iPSYCH, GERA; such approaches have been extensively used to elucidate the epidemiology of major depression), or a combination of methods (deCODE, UK Biobank) whereas ~56% of cases were from 23andMeD (via self-report)[28]. Multiple lines of genetic evidence supported conducting meta-analysis of these seven cohorts (e.g., out-of-sample prediction, sign tests, and genetic correlations).

However, our approach may be controversial to some readers given the unconventional reliance on self-report of major depression. We would reframe the issue: we hypothesize that brief methods of assessing major depression are informative for the genetics of MDD. We present a body of results that are consistent with this hypothesis. Even if unconventional, our hypothesis is testable and falsifiable, and we invite and welcome empirical studies to further support or refute this hypothesis.

Our results lead us to draw some broad conclusions. First, major depression is a brain disorder. Although this is not unexpected, some past models of MDD have had little or no place for heredity or biology. The genetic results best match gene expression patterns in prefrontal and anterior cingulate cortex, anatomical regions that show differences between MDD cases and controls. The genetic findings implicated neurons (not microglia or astrocytes), and we anticipate more detailed cellular localization when sufficient single-cell and single-nuclei RNA-seq datasets become available[67].

Second, the genetic associations for major depression (as with schizophrenia)[46] tend to occur in genomic regions conserved across a range of placental mammals. Conservation suggests important functional roles. Notably, our analyses did not implicate exons or coding regions.

Third, the results also implicated developmental gene regulatory processes. For instance, the genetic findings pointed at the splicing regulator *RBFOX1* (the presence of two independent genetic associations in *RBFOX1* strongly suggests that it is the relevant gene). Gene set analyses implicated genes containing binding sites to the protein product of *RBFOX1*, and this gene set is also significantly enriched for rare exonic variation in autism and schizophrenia[56,57]. These analyses highlight the potential importance of splicing to generate alternative isoforms; risk for major depression may be mediated not by changes in isolated amino acids but rather by changes in the proportions of isoforms coming from a gene, given that isoforms often have markedly different biological functions[68,69]. These convergent results provide possible clues of a biological mechanism common to multiple severe psychiatric disorders that merits future research.

Fourth, in the most extensive analysis of the genetic "connections" of major depression with a wide range of disorders, diseases, and human traits, we found significant positive genetic correlations with measures of body mass and negative genetic correlations with years of education, while showing no evidence of genetic correlation with IQ. MR analysis results are consistent with both BMI and years of education being causal, or correlated with causal, risk factors for major depression, and our results provide hypotheses and motivation for more detailed prospective studies, as currently available data may not provide insight about the fundamental driver or drivers of causality. The underlying mechanisms are likely more complex as it is difficult to envision how genetic variation in educational attainment or body mass alters risk for MDD without invoking an additional mechanistic component. While the significant MR analyses need further investigations to fully understand, the negative MR results provide important evidence that there is not a direct causal relationship between MDD and subsequent changes in body mass or education years. If such associations are observed in epidemiological or clinical samples, then it is likely not MDD but something correlated with MDD that drives the association.

Fifth, we found significant positive correlations of major depression with all psychiatric disorders that we evaluated, including disorders prominent in childhood. This pattern of results indicates that the current classification scheme for major psychiatric disorders does not align well with the underlying genetic basis of these disorders. Currently, only schizophrenia has a sufficient number of genome-wide significant loci to conduct MR analysis, but the bidirectionally significant MR results are consistent a shared biological basis for major depression and schizophrenia.

The dominant psychiatric nosological systems were principally designed for clinical utility, and are based on data that emerge during human interactions (i.e., observable signs and reported symptoms) and not objective measurements of pathophysiology. MDD is frequently comorbid with other psychiatric disorders, and the phenotypic comorbidity has an underlying structure that reflects shared origins (as inferred from factor analyses and twin studies)[70-73]. Our genetic results add to this knowledge: major depression is not a discrete entity at any level of analysis. Rather, our data strongly suggest the existence of biological processes common to major depression and schizophrenia (and likely, other psychiatric disorders).

Finally, as expected, we found that major depression had modest h^2_{SNP} (8.7%) as it is a complex malady with both genetic and environmental determinants. We found that major depression has a very high genetic correlation with proxy

measures that can be briefly assessed. Lifetime major depressive disorder requires a constellation of signs and symptoms whose reliable scoring requires an extended interview with a trained clinician. However, the common variant genetic architecture of lifetime major depression in these seven cohorts (containing many subjects medically treated for MDD) has strong overlap with that of current depressive symptoms in general community samples. Similar relations of clinically-defined ADHD or autism with quantitative genetic variation in the population have been reported[74,75]. The "disorder versus symptom" relationship has been debated extensively[76], but our data indicate that the common variant genetic overlap is very high. This finding has important implications.

One implication is for future genetic studies. In a first phase, it should be possible to elucidate the bulk of the common variant genetic architecture of MDD using a cost-effective shortcut – large studies of genotyped individuals who complete online self-report assessments of lifetime MDD (a sample size approaching 1 million MDD cases may be achievable by 2020). Use of online assessment could allow for recording of a broad range of phenotypes including comorbidities and putative environmental exposures, but the key feature being large samples with consistently assessed measures. In a second phase, with a relatively complete understanding of the genetic basis of major depression, one could then evaluate smaller samples of carefully phenotyped individuals with MDD to understand the clinical importance of the genetic results. Subsequent empirical studies may show that it is possible to stratify MDD cases at first presentation to identify individuals at high risk for recurrence, poor outcome, poor treatment response, or who might subsequently develop a psychiatric disorder requiring alternative pharmacotherapy (e.g., schizophrenia or bipolar disorder). This could form a cornerstone of precision medicine in psychiatry.

In summary, this GWA meta-analysis of 135,438 MDD and major depression cases and 344,901 controls identified 44 loci. An extensive set of companion analyses provide insights into the nature of MDD as well as its neurobiology, therapeutic relevance, and genetic and biological interconnections to other psychiatric disorders. Comprehensive elucidation of these features is the primary goal of our genetic studies of MDD.

URLs

1000 Genomes Project multi-ancestry imputation panel, https://mathgen.stats.ox.ac.uk/impute/data_download_1000G_phase1_integrated.html

23andMe privacy policy https://www.23andme.com/en-eu/about/privacy

Bedtools, https://bedtools.readthedocs.io

dbGaP, https://www.ncbi.nlm.nih.gov/gap

Genotype-based checksums for relatedness determination, http://www.broadinstitute.org/~sripke/share_links/checksums_download

GSMR, http://cnsgenomics.com/software/gsmr/

GTEx, http://www.gtexportal.org/home/datasets

GTMapTool, http://infochim.u-strasbg.fr/mobyle-cgi/portal.py#forms::gtmaptool

LD-Hub, http://ldsc.broadinstitute.org

PGC website, http://www.med.unc.edu/pgc

NIH NeuroBiobank, https://neurobiobank.nih.gov

PGC "ricopili" GWA pipeline, https://github.com/Nealelab/ricopili

SMR, http://cnsgenomics.com/software/smr/#Overview

TWAS, http://gusevlab.org/projects/fusion/

UK Biobank, http://www.ukbiobank.ac.uk

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Competing Financial Interests

Aartjan TF Beekman: Speakers bureaus of Lundbeck and GlaxoSmithKline. Greg Crawford: Co-founder of Element Genomics. Enrico Domenici: Employee of Hoffmann-La Roche at the time this study was conducted, consultant to Roche and Pierre-Fabre. Nicholas Eriksson: Employed by 23andMe, Inc. and owns stock in 23andMe, Inc. David Hinds: Employee of and own stock options in 23andMe, Inc. Sara Paciga: Employee of Pfizer, Inc. Craig L Hyde: Employee of Pfizer, Inc. Ashley R Winslow: Former employee and stockholder of Pfizer, Inc. Jorge A Quiroz: Employee of Hoffmann-La Roche at the time this study was conducted. Hreinn Stefansson: Employee of deCODE Genetics/AMGEN. Kari Stefansson: Employee of deCODE Genetics/AMGEN. Stacy Steinberg: Employee of deCODE Genetics/AMGEN. Patrick F Sullivan: Scientific advisory board for Pfizer Inc and an advisory committee for Lundbeck. Thorgeir E Thorgeirsson: Employee of deCODE Genetics/AMGEN. Chao Tian: Employee of and own stock options in 23andMe, Inc.

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FIG. LEGENDS FOR MAIN TEXT

Fig. 1: ***Results of GWA meta-analysis of seven cohorts for major depression.*** *(a) Relation between adding cohorts and number of genome-wide significant genomic regions (before the rigorous vetting used to define the final 44 regions). Beginning with the largest cohort (#1 on the x-axis), added the next largest cohort (#2) until all cohorts were included (#7). The number next to each point shows the total effective sample size equivalent to sample size where the numbers of cases and controls are equal. (b) Association test quantile-quantile plot showing a marked departure from a null model of no associations (y-axis truncated 10^{-12}). (c) Manhattan plot with x-axis showing genomic position (chr1-chr22 plus chrX), and the y-axis showing statistical significance as $-log_{10}(P)$ t-statistic; threshold for significance accounting for multiple testing shown by horizontal line. Association test from meta-analysis of 135,458 major depression cases and 344,901 controls. The red line shows the genome-wide significance threshold ($P=5x10^{-8}$).*

Fig. 2: ***Genetic risk score (GRS) prediction analyses into PGC29 MDD target samples.*** *(**a**) Variance explained (liability scale) based on different discovery samples for three target samples: PGC29 (16,823 cases, 25,632 controls), iPSYCH (a nationally representative sample of 18,629 cases and 17,841 controls,) and a clinical cohort from Münster not included in the GWA analysis (845 MDD inpatient cases, 834 controls). PGC29-LOO: Target sample is one of the PGC29 samples, with discovery sample the remaining 28 PGC29 samples, hence, leave-one-out. (**b**) Odds ratios of major depression per GRS decile relative to the first decile for iPSYCH and PGC29 target samples. (**c**) Odds ratios of major depression in GRS standard deviation (SD): 3,950 early onset vs 3,950 late onset cases earlier age at onset; 4,958 severe vs 3,976 moderate cases defined by count of endorsed MDD symptom criteria; 5,574 cases recurrent MDD vs 12,968 single episode cases; severity defined as chronic/unremitting MDD 610 "Stage IV" cases vs 499 "Stage II" or 332 first-episode MDD [77] used the NESDA sample from PGC29. Error bars represent 95% confidence intervals. Logistic regression association test p-values in the target sample for GRS generated from SNPs with p-value < 0.05 in the discovery sample.*

Fig. 3: *Comparisons of the major depression GWA meta-analysis. (**a**) Enrichment in bulk tissue mRNA-seq from GTEx; t-statistic, sample sizes in GTEx range from N=75-564. Threshold for significance accounting for multiple testing shown by vertical line. (**b**) Major depression results and enrichment in three major brain cell types; t-statistic; threshold for significance accounting for multiple testing shown by horizontal line. Sample sizes vary as these data are aggregated from many different sources. (**c**) Partitioned LDSC to evaluate enrichment of the major depression GWA findings in over 50 functional genomic annotations (**Supplementary Table 8**); enrichment statistic; threshold for significance accounting for multiple testing given by horizontal dashed line. Sample sizes vary as these data are aggregated from many different sources.*

Fig. 4: *Generative topographic mapping of the 19 significant pathway results. The average position of each pathway on the map is represented by a point. The map is colored by the $-log_{10}(P)$ obtained using MAGMA. The X and Y coordinates result from a kernel generative topographic mapping algorithm (GTM) that reduces high dimensional gene sets to a two-dimensional scatterplot by accounting for gene overlap between gene sets. Each point represents a gene set. Nearby points are more similar in gene overlap than more distant points. The color surrounding each point (gene set) indicates significance per the scale on the right. The significant pathways (**Supplementary Table 11**) fall into nine main clusters as described in the text.*

Table 1. Brief description of the seven MDD or major depression cohorts used in the meta-analysis

Sample	Country	Case ascertainment	Cases	Controls
PGC29[13, a]	Various	Structured diagnostic interviews[b]	16,823	25,632
deCODE[13]	Iceland	National inpatient electronic records	1,980	9,536
GenScotland[78,79]	UK	Structured diagnostic interview	997	6,358
GERA[80]	USA	Kaiser Permanente Northern California Healthcare electronic medical records (1995-2013)	7,162	38,307
iPSYCH[81]	Denmark	National inpatient electronic records	18,629	17,841
UK Biobank[82] (Pilot data release)	UK	From self-reported MDD symptoms or treatment or electronic records[69]	14,260	15,480
23andMeD[28] (Discovery sample)[c]	USA	Self-reported diagnosis or treatment for clinical depression by a medical professional	75,607	231,747
Total			**135,458**	**344,901**

a: 19 additional samples to the 10 samples published in the first PGC-MDD paper[13].
b: One sample used natural language processing of electronic medical records followed by expert diagnostic review.
c: In Hyde et al.[28] SNPs in 15 genomic regions met genome-wide significance in the combined discovery and replication samples, and 11 regions achieved genome-wide significance in the discovery sample made available to the research community and used here. More details are provided in ***Supplementary Tables 1-3***.

823 ***Table 2. 44 significantly associated genomic regions in meta-analysis of 135,458 major depression cases and 344,901 controls***

Chr	Region (Mb)	SNP	Location-bp	P	A1/2	OR-	SE(log)	Frq	Pre	Gene Context
1	8.390-8.895	rs159963	8,504,421	3.2E-08	A/C	0.97	0.0049	0.56	H,S	*[RERE]; SLC45A1,100194*
1	72.511-73.059	rs1432639	72,813,218	4.6E-15	A/C	1.04	0.0050	0.63	H	*NEGR1,-64941*
1	73.275-74.077	rs12129573	73,768,366	4.0E-12	A/C	1.04	0.0050	0.37	S	*LINC01360,-3486*
1	80.785-80.980	rs2389016	80,799,329	1.0E-08	T/C	1.03	0.0053	0.28	H	
1	90.671-90.966	rs4261101	90,796,053	1.0E-08	A/G	0.97	0.0050	0.37		
1	197.343-197.864	rs9427672	197,754,741	3.1E-08	A/G	0.97	0.0058	0.24		*DENND1B,-10118*
2	57.765-58.485	rs11682175	57,987,593	4.7E-09	T/C	0.97	0.0048	0.52	H,S	*VRK2,-147192*
2	156.978-157.464	rs1226412	157,111,313	2.4E-08	T/C	1.03	0.0059	0.79		*[LINC01876]; NR4A2,69630; GPD2,-180651*
3	44.222-44.997	chr3_44287760_I	44,287,760	4.6E-08	I/D	1.03	0.0051	0.34	T	*[TOPAZ1]; TCAIM,-91850; ZNF445,193501*
3	157.616-158.354	rs7430565	158,107,180	2.9E-09	A/G	0.97	0.0048	0.58	H	*[RSRC1]; LOC100996447,155828; MLF1,-*
4	41.880-42.189	rs34215985	42,047,778	3.1E-09	C/G	0.96	0.0063	0.24		*[SLC30A9]; LINC00682,-163150;*
5	87.443-88.244	chr5_87992715_I	87,992,715	7.9E-11	I/D	0.97	0.0050	0.58	H	*LINC00461,-12095; MEF2C,21342*
5	103.672-104.092	chr5_103942055_D	103,942,055	7.5E-12	I/D	1.03	0.0048	0.48	C	
5	124.204-124.328	rs116755193	124,251,883	7.0E-09	T/C	0.97	0.0050	0.38		*LOC101927421,-120640*
5	164.440-164.789	rs11135349	164,523,472	1.1E-09	A/C	0.97	0.0048	0.48	H	
5	166.977-167.056	rs4869056	166,992,078	6.8E-09	A/G	0.97	0.0050	0.63		*[TENM2]*
6	27.738-32.848	rs115507122	30,737,591	3.3E-11	C/G	0.96	0.0063	0.18	S	*extended MHC*
6	99.335-99.662	rs9402472	99,566,521	2.8E-08	A/G	1.03	0.0059	0.24		*FBXL4,-170672; C6orf168,154271*
7	12.154-12.381	rs10950398	12,264,871	2.6E-08	A/G	1.03	0.0049	0.41		*[TMEM106B]; VWDE,105637*
7	108.925-109.230	rs12666117	109,105,611	1.4E-08	A/G	1.03	0.0048	0.47		
9	2.919-3.009	rs1354115	2,983,774	2.4E-08	A/C	1.03	0.0049	0.62	H	*PUM3,-139644; LINC01231,-197814*
9	11.067-11.847	rs10959913	11,544,964	5.1E-09	T/G	1.03	0.0057	0.76		
9	119.675-119.767	rs7856424	119,733,595	8.5E-09	T/C	0.97	0.0053	0.29		*[ASTN2]*
9	126.292-126.735	rs7029033	126,682,068	2.7E-08	T/C	1.05	0.0093	0.07		*[DENND1A]; LHX2,-91820*
10	106.397-106.904	rs61867293	106,563,924	7.0E-10	T/C	0.96	0.0061	0.20	H	*[SORCS3]*
11	31.121-31.859	rs1806153	31,850,105	1.2E-09	T/G	1.04	0.0059	0.22		*[DKFZp686K1684]; [PAUPAR]; ELP4,44032;*
12	23.924-24.052	rs4074723	23,947,737	3.1E-08	A/C	0.97	0.0049	0.41		*[SOX5]*
13	44.237-44.545	rs4143229	44,327,799	2.5E-08	A/C	0.95	0.0091	0.92		*[ENOX1]; LACC1,-125620; CCDC122,82689*
13	53.605-54.057	rs12552	53,625,781	6.1E-19	A/G	1.04	0.0048	0.44	H	*[OLFM4]; LINC01065,80099*
14	41.941-42.320	rs4904738	42,179,732	2.6E-09	T/C	0.97	0.0049	0.57		*[LRFN5]*
14	64.613-64.878	rs915057	64,686,207	7.6E-10	A/G	0.97	0.0049	0.42		*[SYNE2]; MIR548H1,-124364; ESR2,7222*
14	75.063-75.398	chr14_75356855_I	75,356,855	3.8E-09	D/I	1.03	0.0049	0.49		*[DLST]; PROX2,-26318; RPS6KL1,13801*
14	103.828-104.174	rs10149470	104,017,953	3.1E-09	A/G	0.97	0.0049	0.49	S	*BAG5,4927; APOPT1,-11340*
15	37.562-37.929	rs8025231	37,648,402	2.4E-12	A/C	0.97	0.0048	0.57	H	
16	6.288-6.347	rs8063603	6,310,645	6.9E-09	A/G	0.97	0.0053	0.65		*[RBFOX1]*
16	7.642-7.676	rs7198928	7,666,402	1.0E-08	T/C	1.03	0.0050	0.62		*[RBFOX1]*

16	13.022-13.119	rs7200826	13,066,833	2.4E-08	T/C	1.03	0.0055	0.25		*[SHISA9]; CPPED1,-169089*
16	71.631-72.849	rs11643192	72,214,276	3.4E-08	A/C	1.03	0.0049	0.41		*PMFBP1,-7927; DHX38,67465;*
17	27.345-28.419	rs17727765	27,576,962	8.5E-09	T/C	0.95	0.0088	0.92		*[CRYBA1]; MYO18A,-69555; NUFIP2,5891*
18	36.588-36.976	rs62099069	36,883,737	1.3E-08	A/T	0.97	0.0049	0.42		*[MIR924HG]*
18	50.358-50.958	rs11663393	50,614,732	1.6E-08	A/G	1.03	0.0049	0.45	O	*[DCC]; MIR4528,-148738*
18	51.973-52.552	rs1833288	52,517,906	2.6E-08	A/G	1.03	0.0054	0.72		*[RAB27B]; CCDC68,50833*
18	52.860-53.268	rs12958048	53,101,598	3.6E-11	A/G	1.03	0.0051	0.33	S	*[TCF4]; MIR4529,-44853*
22	40.818-42.216	rs5758265	41,617,897	7.6E-09	A/G	1.03	0.0054	0.28	H,S	*[L3MBTL2]; EP300-AS1,-24392; CHADL,7616*

Chr (chromosome) and Region (boundaries in Mb, hg19) are shown, defined by locations of SNPs with $P<1x10^{-5}$ and LD $r^2 > 0.1$ with the most associated SNP (logistic regression; lowest P-value in region listed not corrected for multiple testing) whose location is given in bp. In three regions a second SNP fulfils the filtering criteria and these were followed up with conditional analyses: Chr1: conditional analysis selects only rs1432639 as significant, with $P=2.0x10^{-4}$ for rs12134600 after fitting rs1432639; Chr5, conditional analysis shows two independent associations selecting rs247910 and rs10514301 as the most associated SNPs; and Chr10 conditional analysis selects only rs61867293 with $P=8.6x10^{-5}$ for rs1021363 after conditioning on rs61867293. For each of the 47 SNPs, there is at least 1 additional genome-wide significant SNP in the cluster of surrounding SNPs with low P-values. Chromosome X was analyzed but had no findings that met genome-wide significance.

Column labels and abbreviations. A1/2 = the two alleles (or insertion-deletion); A1 was tested for association, and its OR (odds ratio) and SE (standard error) are shown. FreqU = frequency of A1 in controls across all cohorts. Entries in the "Prev" column indicate which of four previous studies identified genome-significant associations in a region. H=Hyde et al.[28], 23andMe GWA of self-reported clinical depression (discovery sample overlaps with this paper); O=Okbay et al.[16], meta-analysis of GWA of MDD, depressive symptoms, psychological well-being and neuroticism (includes many PGC29 samples); S=PGC report on 108 schizophrenia-associated loci[19]; and C=CHARGE consortium meta-analysis of depressive symptoms[14]. Gene context: distances between the Peak SNP and the closest genes are shown. Brackets indicate that the Peak SNP was within that gene. The closest genes upstream (taking strand into account, as a negative number indicating distance in bp between Peak SNP and the nearest gene boundary) and downstream (positive distance in bp) are also shown, if there is a flanking gene within 200 kb. The name of the closest gene is bolded. Note that it is generally not known whether the associated SNPs have biological effects on these or other more distant genes.

Table 3. LDSC genetic correlations of MDD with other disorders, diseases, and human traits

Trait	r_g	SE	FDR	h^2_{SNP}	PMID
Depressive symptoms, CHARGE	0.91	0.123	3.2E-12	0.04	23290196
Depressive symptoms, SSGAC	0.98	0.034	1.3E-176	0.05	27089181
ADHD (iPSYCH-PGC)	0.42	0.033	6.1E-36	0.24	submitted
Anorexia nervosa	0.13	0.028	7.1E-05	0.55	24514567
Anxiety disorders	0.80	0.140	2.0E-07	0.06	26857599
Autism spectrum disorders (iPSYCH-PGC)	0.44	0.039	8.4E-28	0.20	submitted
Bipolar disorder	0.32	0.034	3.3E-19	0.43	21926972
Schizophrenia	0.34	0.025	7.7E-40	0.46	25056061
Smoking, ever vs never	0.29	0.038	7.0E-13	0.08	20418890
Daytime sleepiness ‡	0.19	0.048	5.7E-04	0.05	0
Insomnia ‡	0.38	0.038	4.0E-22	0.13	0
Tiredness	0.67	0.037	6.2E-72	0.07	28194004
Subjective well-being	-0.65	0.035	7.5E-76	0.03	27089181
Neuroticism	0.70	0.031	2.5E-107	0.09	27089181
College completion	-0.17	0.034	6.7E-06	0.08	23722424
Years of education	-0.13	0.021	1.6E-08	0.13	27225129
Body fat	0.15	0.038	6.5E-04	0.11	26833246
Body mass index	0.09	0.026	3.6E-03	0.19	20935630
Obesity class 1	0.11	0.029	1.6E-03	0.22	23563607
Obesity class 2	0.12	0.033	3.0E-03	0.18	23563607
Obesity class 3	0.20	0.053	1.6E-03	0.12	23563607
Overweight	0.13	0.030	1.4E-04	0.11	23563607
Waist circumference	0.11	0.024	8.2E-05	0.12	25673412
Waist-to-hip ratio	0.12	0.030	2.9E-04	0.11	25673412
Triglycerides	0.14	0.028	1.0E-05	0.17	20686565
Age at menarche	-0.14	0.023	6.3E-08	0.20	25231870
Age of first birth	-0.29	0.029	6.1E-22	0.06	27798627
Fathers age at death	-0.28	0.058	3.0E-05	0.04	27015805
Number of children ever born	0.13	0.036	2.4E-03	0.03	27798627
Coronary artery disease	0.12	0.027	8.2E-05	0.08	26343387
Squamous cell lung cancer	0.26	0.075	3.6E-03	0.04	27488534

All genetic correlations (r_g) estimated using bivariate LDSC applied to major depression GWA results are in ***Supplementary Table 12***. Shown above are the r_g of major depression with false discovery rate (FDR) < 0.01 (FDR estimated for 221 genetic correlations, H_0: r_g=0). Thematically related traits are indicated by shading. iPSYCH is a nationally representative cohort based on blood spots collected at birth. Within iPSYCH, r_g with ADHD was 0.58 (SE 0.050) and 0.51 (SE 0.07) with ASD – these are larger than those listed above, and inconsistent with artefactual correlations. h^2_{SNP} is shown to aid interpretation as high r_g in the context of high h^2_{SNP} is more noteworthy than when h^2_{SNP} is low. PMID is PubMed article identifier.

‡ Self-reported daytime sleepiness and insomnia from UK Biobank excluding subjects with major depression, other psychiatric disorders (bipolar disorder, schizophrenia, autism, intellectual disability), shift workers, and those taking hypnotics.

ONLINE METHODS

PGC29 cohort. Our analysis was anchored in a GWA mega-analysis of 29 samples of European-ancestry (16,823 MDD cases and 25,632 controls). ***Supplementary Table 1*** summarizes the source and inclusion/exclusion criteria for cases and controls for each sample. All PGC29 samples passed a structured methodological review by MDD assessment experts (DF Levinson and KS Kendler). Cases were required to meet international consensus criteria (DSM-IV, ICD-9, or ICD-10)[83-85] for a lifetime diagnosis of MDD established using structured diagnostic instruments from assessments by trained interviewers, clinician-administered checklists, or medical record review. All cases met standard criteria for MDD, were directly interviewed (28/29 samples) or had medical record review by an expert diagnostician (1/29 samples), and most were ascertained from clinical sources (19/29 samples). Controls in most samples were screened for the absence of lifetime MDD (22/29 samples), and randomly selected from the population.

Additional cohorts. We critically evaluated six independent, European-ancestry cohorts (118,635 cases and 319,269 controls). ***Supplementary Table 2*** summarizes the source and inclusion/exclusion criteria for cases and controls for each cohort. These cohorts used a range of methods for assessing MDD or major depression. Most studies included here applied otherwise typical inclusion and exclusion criteria for both cases and controls (e.g., excluding cases with lifetime bipolar disorder or schizophrenia and excluding controls with major depression).

Cohort comparability. ***Supplementary Table 3*** summarizes the numbers of cases and controls in PGC29 and the six additional cohorts. The most direct and important way to evaluate the comparability of these cohorts for a GWA meta-analysis is using SNP genotype data. [22,24] We used LD score (LDSC) regression (described below) to estimate h^2_{SNP} for each cohort (***Supplementary Table 3*** and ***Supplementary Fig. 1***), and r_g for all pairwise combinations of the cohorts (***Supplementary Table 3b***), and to demonstrate no evidence of sample overlap. We used leave-one-sample-out genetic risk scores (GRS) finding significant differences in case-control GRS distributions of the left-out-sample for all-but-one PGC29 samples (***Supplementary Table 4***). For full details of the cohort comparability analyses including GRS analyses see the ***Supplementary Note***. In GRS analyses the discovery sample is the GWA sample that provides the allelic-weightings for each SNP used to generate a sum score for each individual in the independent target sample.

Genotyping and quality control. Genotyping procedures can be found in the primary reports for each cohort (summarized in ***Supplementary Table 3***). Individual genotype data for all PGC29 samples, GERA, and iPSYCH were processed using the PGC "ricopili" pipeline (URLs) for standardized quality control, imputation, and analysis[19]. The cohorts from deCODE, Generation Scotland, UK Biobank, and 23andMeD were processed by the collaborating research teams using comparable procedures. SNPs and insertion-deletion polymorphisms were imputed using the 1000 Genomes Project multi-ancestry reference panel (URLs)[86]. More detailed information on sample QC is provided in the ***Supplementary Note***.

Linkage disequilibrium (LD) score regression (LDSC)[22,24] was used to estimate h^2_{SNP} from GWA summary statistics. Estimates of h^2_{SNP} on the liability scale depend on the assumed lifetime prevalence of MDD in the population (K), and we assumed K=0.15 but also evaluated a range of estimates of K to explore sensitivity including 95% confidence intervals (***Supplementary Fig. 1***). LDSC bivariate genetic correlations attributable to genome-wide SNPs (r_g) were estimated across all MDD and major depression cohorts and between the full meta-analyzed cohort and other traits and disorders.

LDSC was also used to partition h^2_{SNP} by genomic features[24,46]. We tested for enrichment of h^2_{SNP} based on genomic annotations partitioning h^2_{SNP} proportional to bp length represented by each annotation. We used the "baseline model" which consists of 53 functional categories. The categories are fully

described elsewhere[46], and included conserved regions[47], USCC gene models (exons, introns, promoters, UTRs), and functional genomic annotations constructed using data from ENCODE [87] and the Roadmap Epigenomics Consortium[88]. We complemented these annotations by adding introgressed regions from the Neanderthal genome in European populations[89] and open chromatin regions from the brain dorsolateral prefrontal cortex. The open chromatin regions were obtained from an ATAC-seq experiment performed in 288 samples (N=135 controls, N=137 schizophrenia, N=10 bipolar, and N=6 affective disorder)[90]. Peaks called with MACS[91] (1% FDR) were retained if their coordinates overlapped in at least two samples. The peaks were re-centered and set to a fixed width of 300bp using the diffbind R package[92]. To prevent upward bias in heritability enrichment estimation, we added two categories created by expanding both the Neanderthal introgressed regions and open chromatin regions by 250bp on each side.

We used LDSC to estimate r_g between major depression and a range of other disorders, diseases, and human traits[22]. The intent of these comparisons was to evaluate the extent of shared common variant genetic architectures in order to suggest hypotheses about the fundamental genetic basis of major depression (given its extensive comorbidity with psychiatric and medical conditions and its association with anthropometric and other risk factors). Subject overlap of itself does not bias r_g. These r_g are mostly based on studies of independent subjects and the estimates should be unbiased by confounding of genetic and non-genetic effects (except if there is genotype by environment correlation). When GWA studies include overlapping samples, r_g remains unbiased but the intercept of the LDSC regression is an estimate of the correlation between association statistics attributable to sample overlap. These calculations were done using the internal PGC GWA library and with LD-Hub (URLs)[60].

Integration of GWA findings to tissue and cellular gene expression. We used partitioned LDSC to evaluate which somatic tissues were enriched for major depression heritability[93]. Gene expression data generated using mRNA-seq from multiple human tissues were obtained from GTEx v6p (URLs). Genes for which <4 samples had at least one read count per million were discarded, and samples with <100 genes with at least one read count per million were excluded. The data were normalized, and a t-statistic was obtained for each tissue by comparing the expression in each tissue with the expression of all other tissues with the exception of tissues related to the tissue of interest (e.g., brain cortex vs all other tissues excluding other brain samples), using sex and age as covariates. A t-statistic was also obtained for each tissue among its related tissue (ex: cortex vs all other brain tissues) to test which brain region was the most associated with major depression, also using sex and age as covariates. The top 10% of the genes with the most extreme t-statistic were defined as tissue specific. The coordinates for these genes were extended by a 100kb window and tested using LD score regression. Significance was obtained from the coefficient z-score, which corrects for all other categories in the baseline model.

Lists of genes specifically expressed in neurons, astrocytes, and oligodendrocytes were obtained from Cahoy et al.[45] As these experiment were done in mice, genes were mapped to human orthologous genes using ENSEMBL. The coordinates for these genes were extended by a 100kb window and tested using LD score regression as for the GTEx tissue specific genes.

We conducted eQTL look-ups of the most associated SNPs in each region and report GWA SNPs in LD (r^2 > 0.8) with the top eQTLs in the following data sets: eQTLGen Consortium (Illumina arrays in whole blood N=14,115, in preparation), BIOS (RNA-seq in whole blood (N=2,116),[94] NESDA/NTR (Affymetrix arrays in whole blood, N=4,896),[95] GEUVADIS (RNA-seq in LCL (N=465),[96] Rosmap (RNA seq in cortex, N= 494)[97], GTEx (RNA-seq in 44 tissues, N>70)[43], and Common Mind Consortium (CMC, prefrontal cortex, Sage Synapse accession syn5650509, N=467)[51].

We used summary-data-based Mendelian randomization (SMR)[49] to identify loci with strong evidence of causality via gene expression and DNA methylation (eQTL and meQTL). SMR analysis is limited to significant cis SNP-expression (FDR < 0.05) and SNPs with MAF > 0.01 at a Bonferroni-corrected pSMR. Due to LD, multiple SNPs may be associated with the expression of a gene, and some SNPs are associated with the expression of more than one gene. Since the aim of SMR is to prioritize variants and genes for subsequent studies, a test for heterogeneity excludes regions that may harbor multiple causal loci (pHET < 0.05; a very conservative threshold). SMR analyses were conducted using eQTLs from eQTLGen Consortium (whole blood), GTEx (11 brain tissues), and Common Mind Consortium[43,51] as well as meQTLs from whole blood[98].

We conducted a transcriptome wide association study[50] using pre-computed expression reference weights for CMC data (5,420 genes with significant cis-SNP heritability) provided with the TWAS/FUSION software. The significance threshold was 0.05/5420.

DNA looping using Hi-C. Dorsolateral prefrontal cortex (Brodmann area 9) was dissected from postmortem samples from three adults of European ancestry (Dr Craig Stockmeier, University of Mississippi Medical Center). Cerebra from three fetal brains were obtained from the NIH NeuroBiobank (URLs; gestation age 17-19 weeks, African ancestry). We used "easy Hi-C" to assess DNA chromatin (looping) interactions (see ***Supplementary Note***).

Gene-wise and pathway analysis. Our approach was guided by rigorous method comparisons conducted by PGC members[55,99]. *P*-values quantifying the degree of association of genes and gene sets with MDD were generated using MAGMA (v1.06)[100]. MAGMA uses Brown's method to combine SNP p-values and account for LD. We used ENSEMBL gene models for 19,079 genes giving a Bonferroni corrected *P*-value threshold of 2.6×10^{-6}. Gene set *P*-values were obtained using a competitive analysis that tests whether genes in a gene set are more strongly associated with the phenotype than other gene sets. We used European-ancestry subjects from 1,000 Genomes Project (Phase 3 v5a, MAF ≥ 0.01)[101] for the LD reference. The gene window used was 35 kb upstream and 10 kb downstream to include regulatory elements.

Gene sets were from two main sources. First, we included gene sets previously shown to be important for psychiatric disorders (71 gene sets; e.g., FMRP binding partners, *de novo* mutations, GWAS top SNPs, ion channels)[57,102,103]. Second, we included gene sets from MSigDB (v5.2)[104] which includes canonical pathways and Gene Ontology gene sets. Canonical pathways were curated from BioCarta, KEGG, Matrisome, Pathway Interaction Database, Reactome, SigmaAldrich, Signaling Gateway, Signal Transduction KE, and SuperArray. Pathways containing between 10-10K genes were included.

To evaluate gene sets related to antidepressants, gene-sets were extracted from the Drug-Gene Interaction database (DGIdb v.2.0)[105] and the Psychoactive Drug Screening Program Ki DB[106] downloaded in June 2016. The association of 3,885 drug gene-sets with major depression was estimated using MAGMA (v1.6). The drug gene-sets were ordered by p-value, and the Wilcoxon-Mann-Whitney test was used to assess whether the 42 antidepressant gene-sets in the dataset (ATC code N06A in the Anatomical Therapeutic Chemical Classification System) had a higher ranking than expected by chance.

One issue is that some gene sets contain overlapping genes, and these may reflect largely overlapping results. The pathway map was constructed using the kernel generative topographic mapping algorithm (k-GTM) as described by Olier et al.[107] GTM is a probabilistic alternative to Kohonen maps: the kernel variant is used when the input is a similarity matrix. The GTM and k-GTM algorithms are implemented in GTMapTool (URLs). We used the Jaccard similarity matrix of FDR-significant pathways as input for the algorithm, where each pathway is encoded by a vector of binary values representing the presence (1) or absence (0) of a gene. Parameters for the k-GTM algorithm are the square root of the number of grid

points (k), the square root of the number of RBF functions (m), the regularization coefficient (l), the RBF width factor (w), and the number of feature space dimensions for the kernel algorithm (b). We set k=square root of the number of pathways, m=square root of k, l=1 (default), w=1 (default), and b=the number of principal components explaining 99.5% of the variance in the kernel matrix. The output of the program is a set of coordinates representing the average positions of pathways on a 2D map. The x and y axes represent the dimensions of a 2D latent space. The pathway coordinates and corresponding MAGMA *P*-values were used to build the pathway activity landscape using the kriging interpolation algorithm implemented in the R gstat package.

Mendelian randomization (MR).[108] We conducted bi-directional MR analysis for four traits: years of education (EDY)[62], body mass index (BMI)[29], coronary artery disease (CAD)[63], and schizophrenia (SCZ)[19]. We denote z as a genetic variant (i.e., a SNP) that is significantly associated with x, an exposure or putative causal trait for y (the disease/trait outcome). The effect size of x on y can be estimated using a two-step least squares (2SLS)[109] approach: $\hat{b}_{xy} = \hat{b}_{zy}/\hat{b}_{zx}$, where $\hat{b}_{zx}$ is the estimated effect size for the SNP-trait association the exposure trait, and $\hat{b}_{zy}$ is the effect size estimated for the same SNP in the GWAS of the outcome trait.

We used generalized summary statistics-based MR (GSMR)[64] to estimate $\hat{b}_{xy}$ and its standard error from multiple SNPs associated with the exposure trait at a genome-wide significance level. We conducted bi-directional GSMR analyses for each pair of traits, and report results after excluding SNPs that fail the HEIDI-outlier heterogeneity test (which is more conservative than excluding SNPs that have an outlying association likely driven by locus-specific pleiotropy). GSMR is more powerful than inverse-weighted MR (IVW-MR) and MR-Egger because it takes account of the sampling variation of both $\hat{b}_{zx}$ and $\hat{b}_{zy}$. GSMR also accounts for residual LD between the clumped SNPs. For comparison, we also conducted IVW-MR and MR-Egger analyses. [110] More details are provided in the ***Supplementary Note***.

Genome build. All genomic coordinates are given in NCBI Build 37/UCSC hg19.

Data availability. The PGC's policy is to make genome-wide summary results public. Summary statistics for a combined meta-analysis of PGC29 with five of the six expanded samples (deCODE, Generation Scotland, GERA, iPSYCH, and UK Biobank) are available on the PGC web site (URLs). Results for 10,000 SNPs for all seven cohorts are also available on the PGC web site.

GWA summary statistics for the Hyde et al. cohort (23andMe, Inc.) must be obtained separately. These can be obtained by qualified researchers under an agreement with 23andMe that protects the privacy of the 23andMe participants. Contact David Hinds (dhinds@23andme.com) to apply for access to the data. Researchers who have the 23andMe summary statistics can readily recreate our results by meta-analyzing the six cohort results file with the Hyde et al. results file from 23andMe.[28]

Availability of genotype data for PGC29 is described in ***Supplementary Table 15***. For the expanded cohorts, interested users should contact the lead PIs of these cohorts (which are separate from the PGC).

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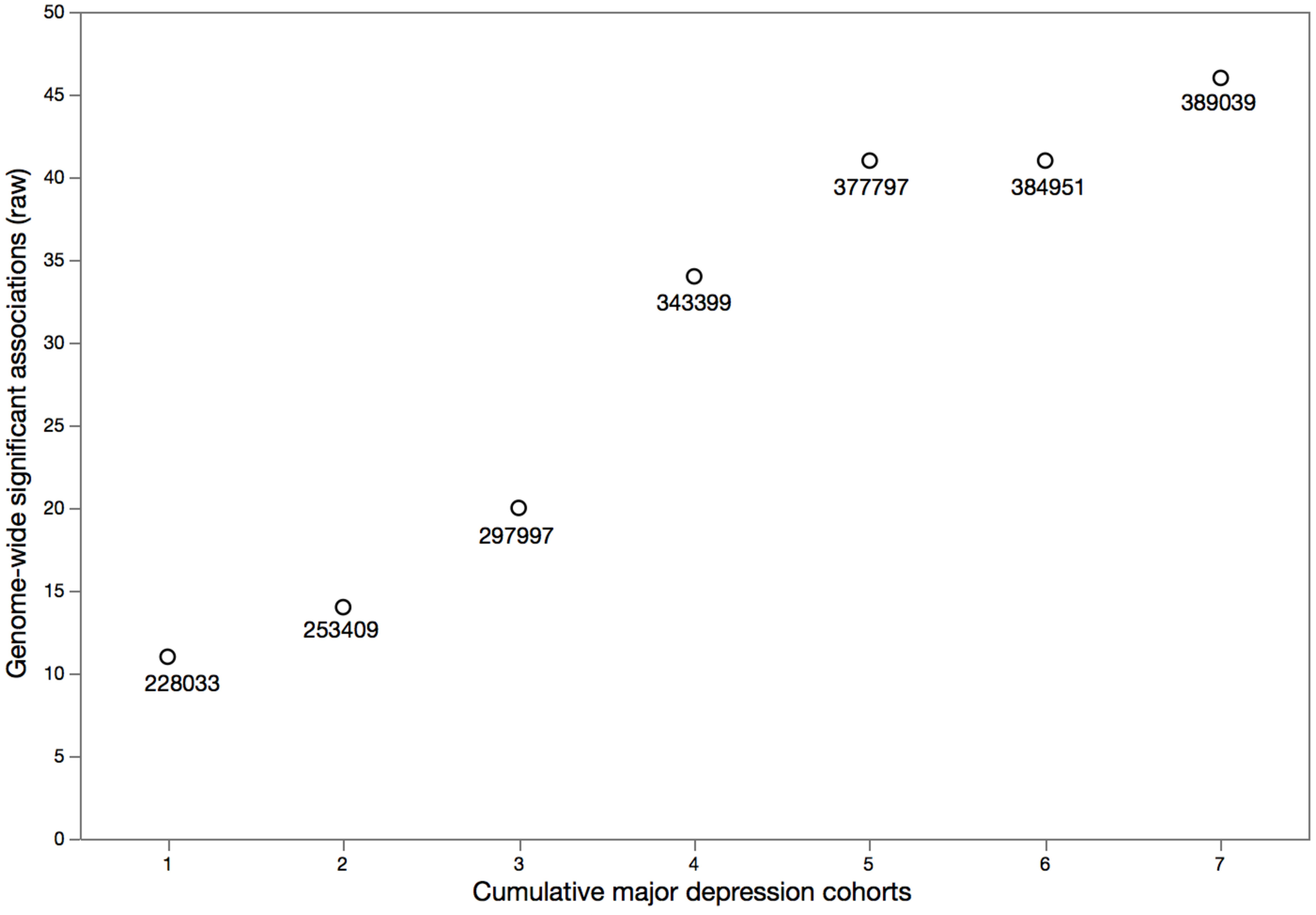
Genome-wide significant associations (raw)
50
45
40
35
30
25
20
15
10
5
0
343399
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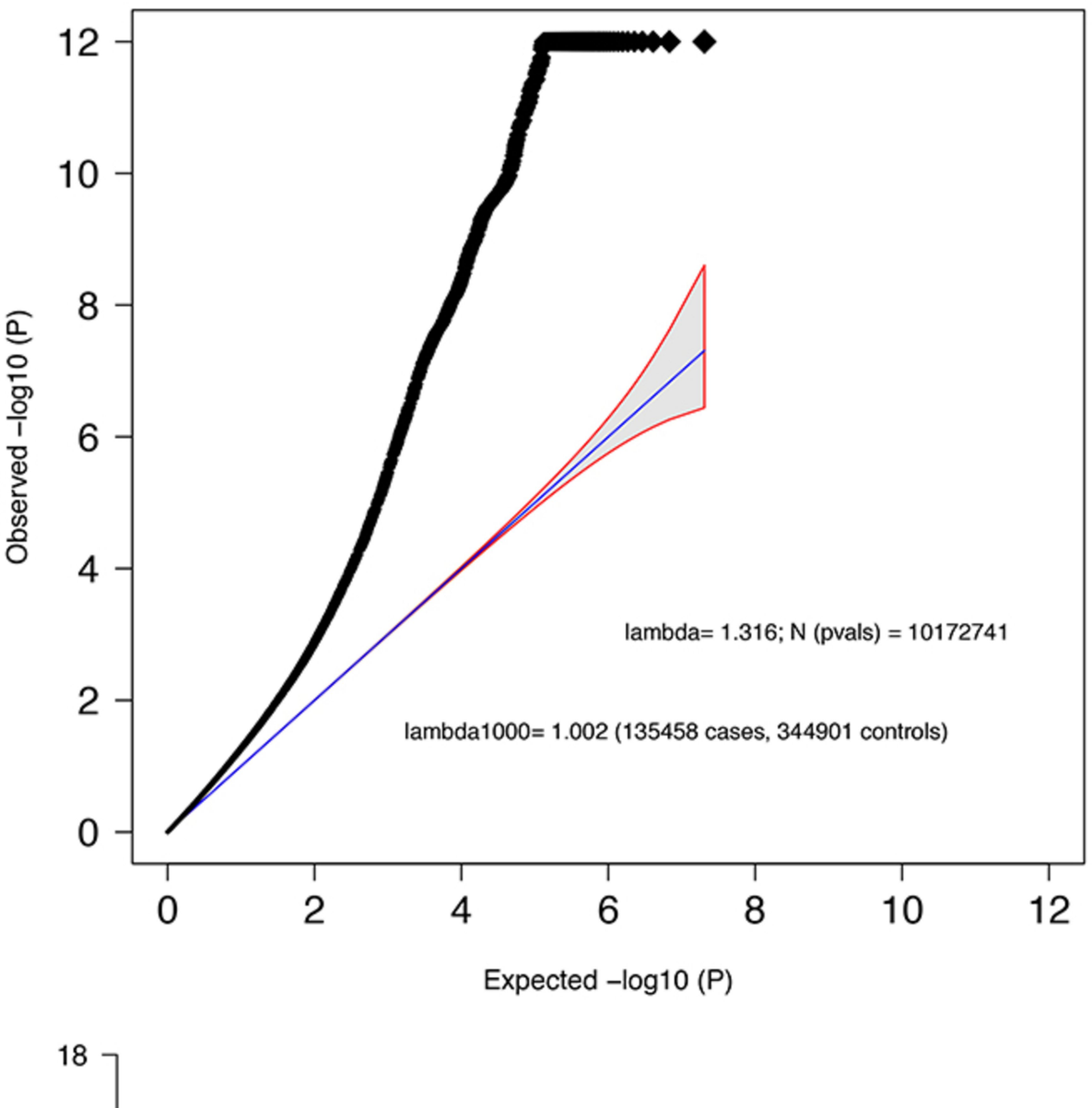
389039
297997
253409
228033
1
2
3
4
5
6
7
Cumulative major depression cohorts


Observed −log10 (P)
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10
8
6
4
2
0
lambda= 1.316; N (pvals) = 10172741
lambda1000= 1.002 (135458 cases, 344901 controls)
0
2
4
6
8
10
12
Expected −log10 (P)

−log10 (p)
18
15
12
9
6
3
1
2
3
4
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14
15
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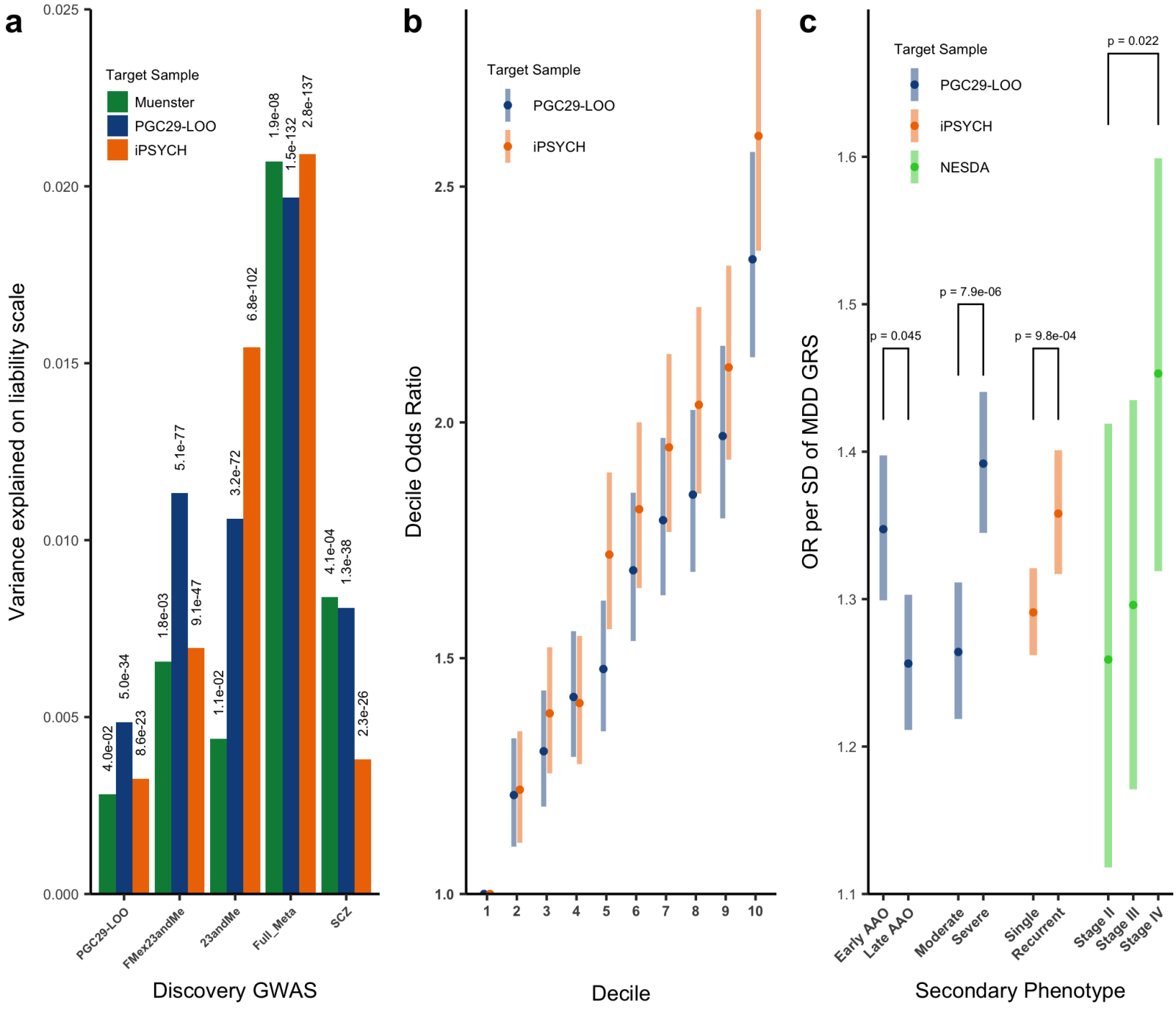
Chromosome


a
Target Sample
Muenster
PGC29-LOO
iPSYCH
Variance explained on liability scale
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0.020
0.015
0.010
0.005
0.000
4.0e-02
5.0e-34
8.6e-23
1.8e-03
5.1e-77
9.1e-47
1.1e-02
3.2e-72
6.8e-102
1.9e-08
1.5e-132
2.8e-137
4.1e-04
1.3e-38
2.3e-26
PGC29-LOO
FMex23andMe
23andMe
Full_Meta
SCZ
Discovery GWAS
b
Target Sample
PGC29-LOO
iPSYCH
Decile Odds Ratio
2.5
2.0
1.5
1.0
1
2
3
4
5
6
7
8
9
10
Decile
c
Target Sample
PGC29-LOO
iPSYCH
NESDA
OR per SD of MDD GRS
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1.5
1.4
1.3
1.2
1.1
p = 0.045
p = 7.9e-06
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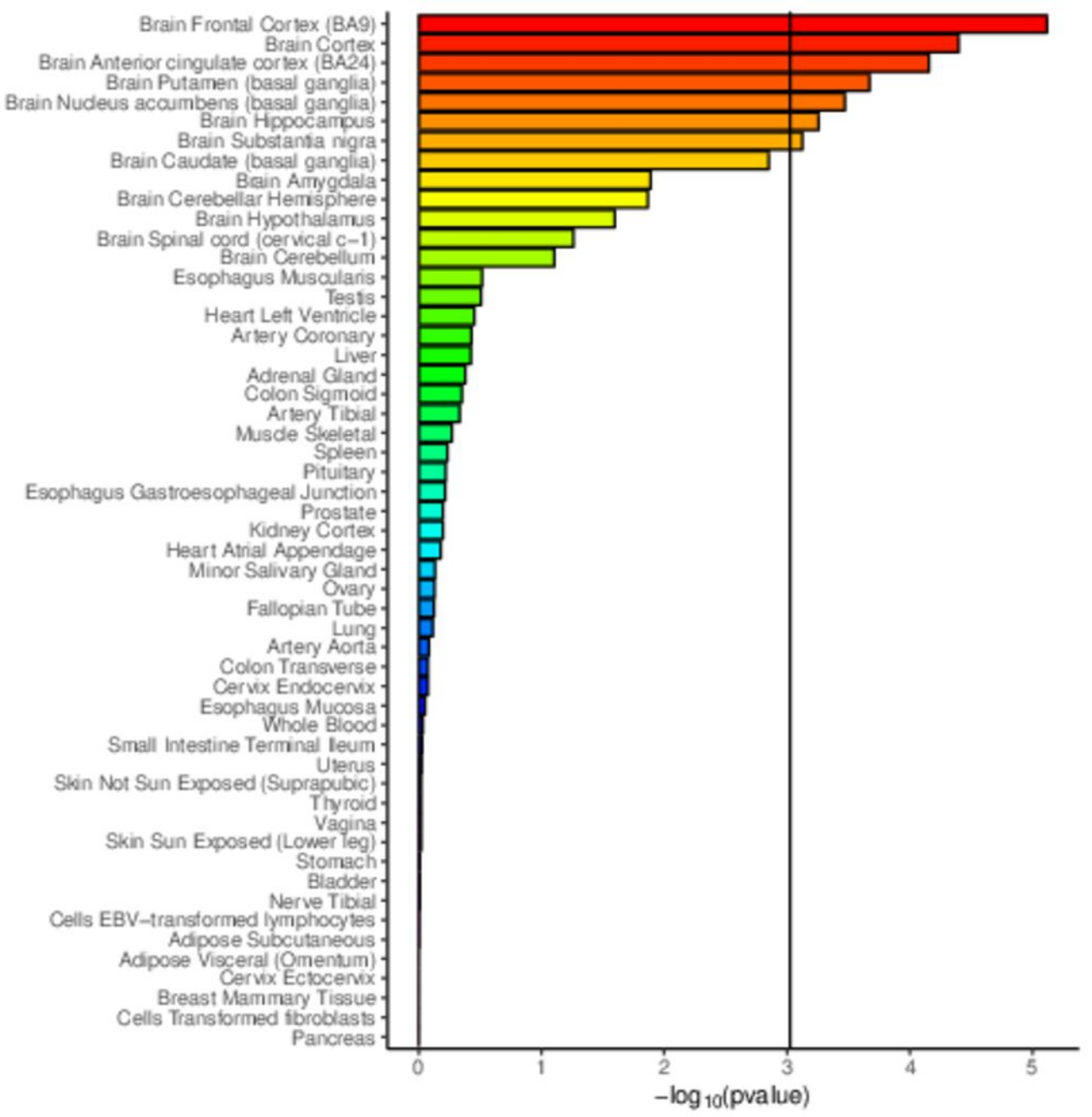
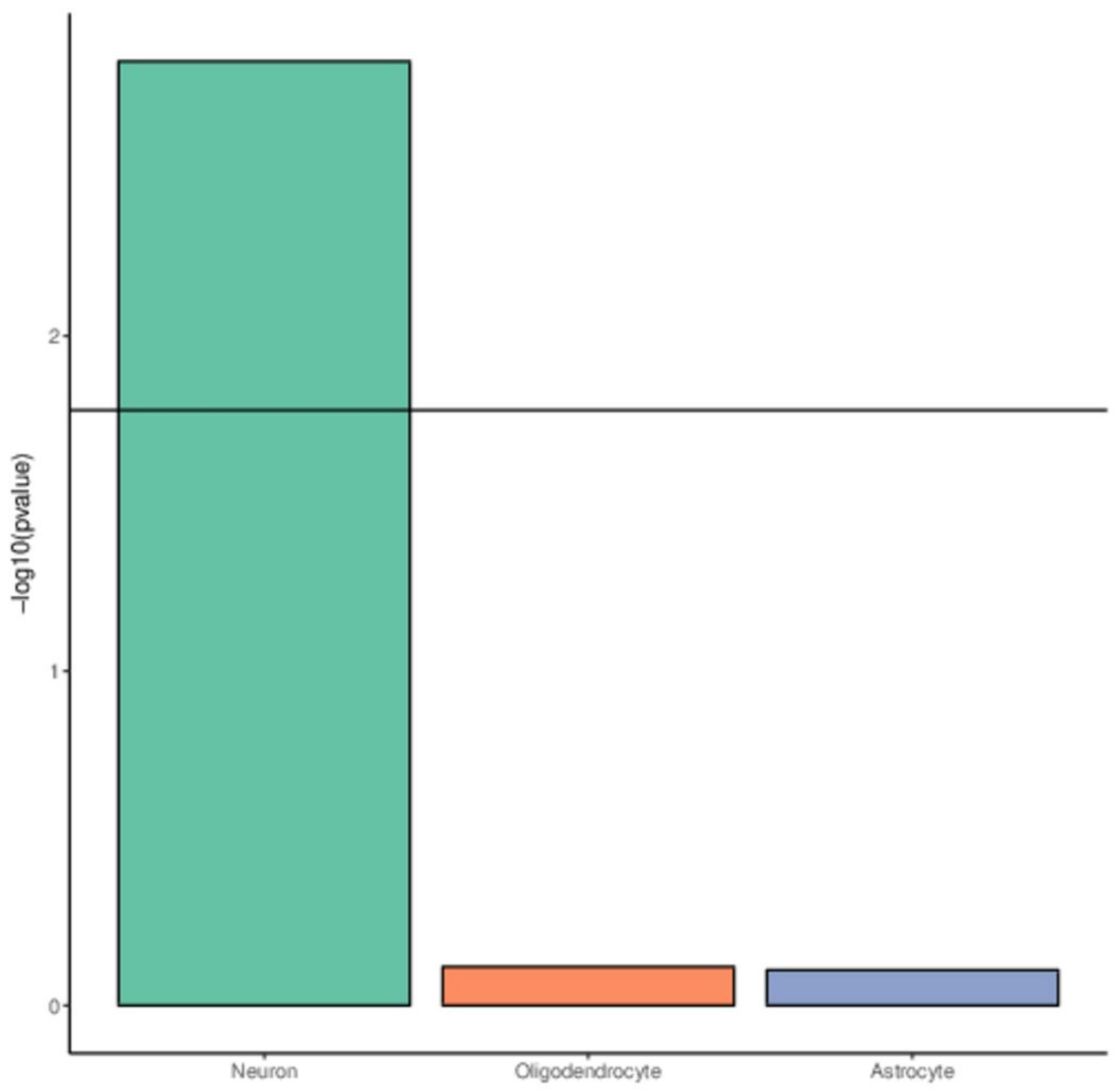
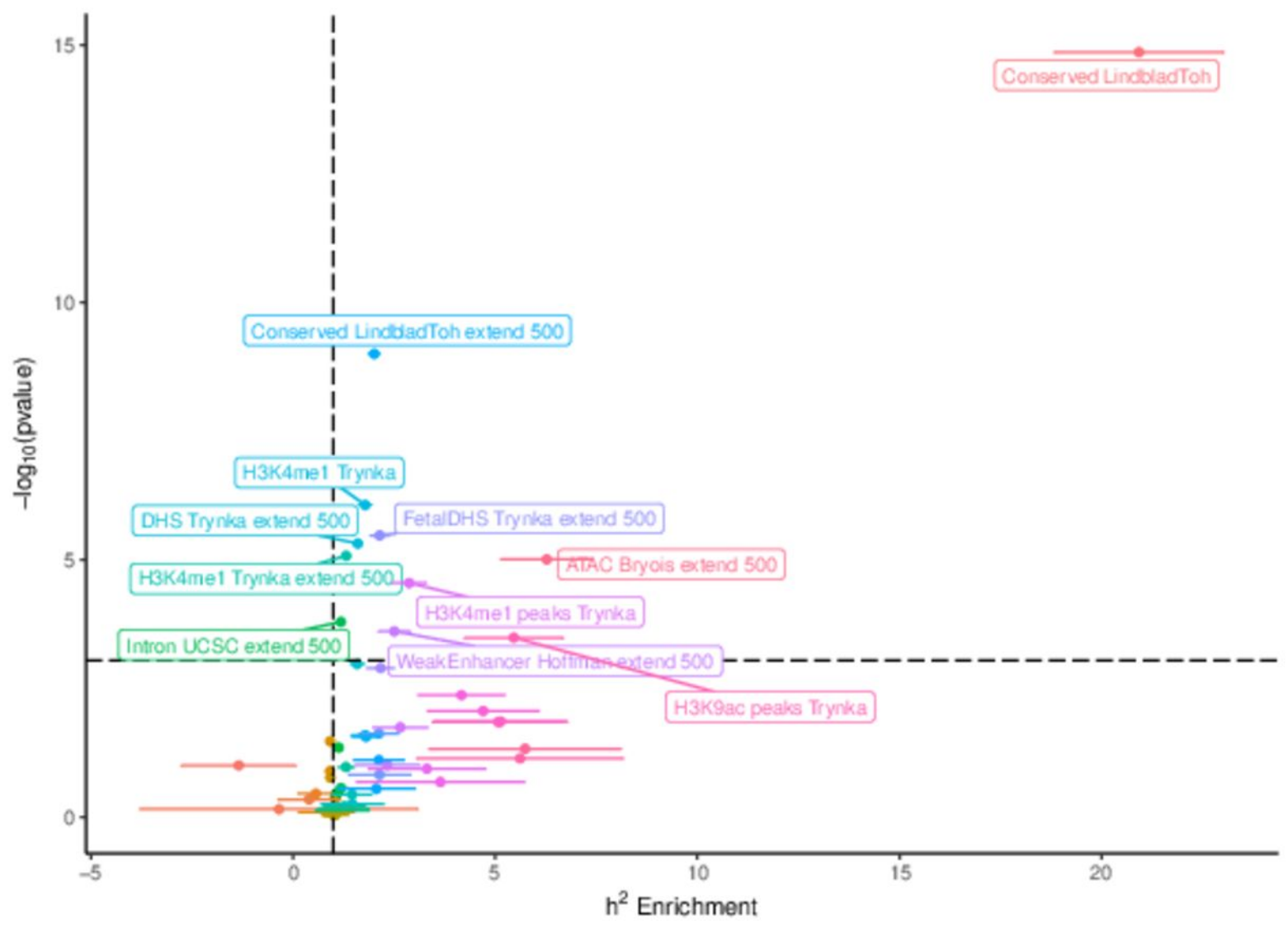
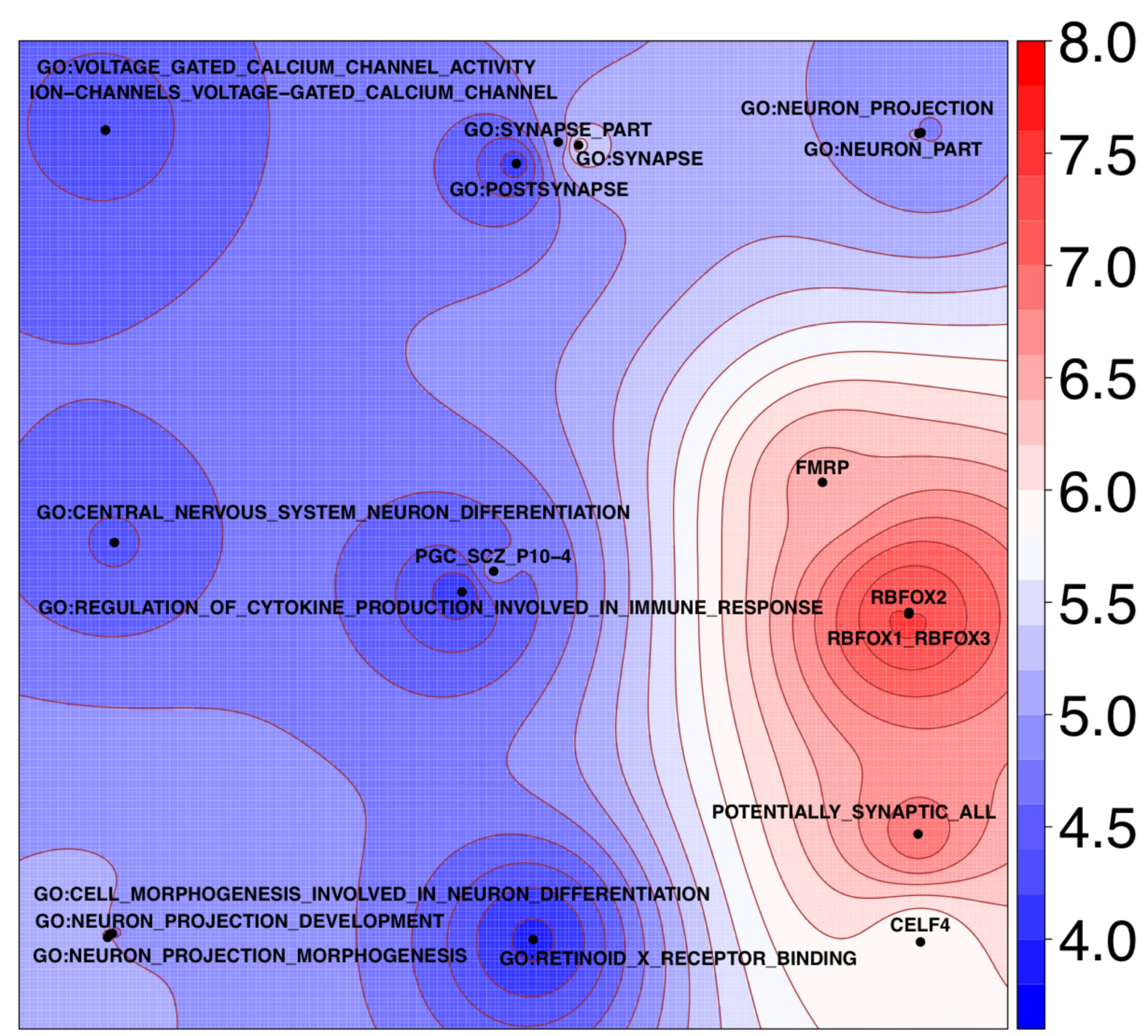
Late AAO
Moderate
Severe
Single
Recurrent
Stage II
Stage III
Stage IV
Secondary Phenotype


Brain Frontal Cortex (BA9)
Brain Cortex
Brain Anterior cingulate cortex (BA24)
Brain Putamen (basal ganglia)
Brain Nucleus accumbens (basal ganglia)
Brain Hippocampus
Brain Substantia nigra
Brain Caudate (basal ganglia)
Brain Amygdala
Brain Cerebellar Hemisphere
Brain Hypothalamus
Brain Spinal cord (cervical c−1)
Brain Cerebellum
Esophagus Muscularis
Testis
Heart Left Ventricle
Artery Coronary
Liver
Adrenal Gland
Colon Sigmoid
Artery Tibial
Muscle Skeletal
Spleen
Pituitary
Esophagus Gastroesophageal Junction
Prostate
Kidney Cortex
Heart Atrial Appendage
Minor Salivary Gland
Ovary
Fallopian Tube
Lung
Artery Aorta
Colon Transverse
Cervix Endocervix
Esophagus Mucosa
Whole Blood
Small Intestine Terminal Ileum
Uterus
Skin Not Sun Exposed (Suprapubic)
Thyroid
Vagina
Skin Sun Exposed (Lower leg)
Stomach
Bladder
Nerve Tibial
Cells EBV−transformed lymphocytes
Adipose Subcutaneous
Adipose Visceral (Omentum)
Cervix Ectocervix
Breast Mammary Tissue
Cells Transformed fibroblasts
Pancreas
0
1
2
3
4
5
−log 10(pvalue)


2
1
0
−log10(pvalue)
Neuron
Oligodendrocyte
Astrocyte


15
10
5
0
−log10(pvalue)
Conserved LindbladToh
Conserved LindbladToh extend 500
H3K4me1 Trynka
DHS Trynka extend 500
FetalDHS Trynka extend 500
ATAC Bryois extend 500
H3K4me1 Trynka extend 500
H3K4me1 peaks Trynka
Intron UCSC extend 500
WeakEnhancer Hoffman extend 500
H3K9ac peaks Trynka
−5
0
5
10
15
20
h2 Enrichment


GO:VOLTAGE_GATED_CALCIUM_CHANNEL_ACTIVITY
ION−CHANNELS_VOLTAGE−GATED_CALCIUM_CHANNEL
GO:SYNAPSE_PART
GO:SYNAPSE
GO:POSTSYNAPSE
GO:NEURON_PROJECTION
GO:NEURON_PART
FMRP
GO:CENTRAL_NERVOUS_SYSTEM_NEURON_DIFFERENTIATION
PGC_SCZ_P10−4
GO:REGULATION_OF_CYTOKINE_PRODUCTION_INVOLVED_IN_IMMUNE_RESPONSE
RBFOX2
RBFOX1_RBFOX3
POTENTIALLY_SYNAPTIC_ALL
GO:CELL_MORPHOGENESIS_INVOLVED_IN_NEURON_DIFFERENTIATION
GO:NEURON_PROJECTION_DEVELOPMENT
GO:NEURON_PROJECTION_MORPHOGENESIS
GO:RETINOID_X_RECEPTOR_BINDING
CELF4
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0